HHS Public Access

Author manuscript

SLAS Discov. Author manuscript; available in PMC 2019 July 01.

Published in final edited form as:

SLAS Discov. 2019 July ; 24(6): 693–700. doi:10.1177/2472555219844569.

A General TR-FRET Assay Platform for High-Throughput Screening and Characterizing Inhibitors of Methyl-Lysine Reader Proteins

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Abstract

Chromatin regulatory complexes localize to specific sites *via* recognition of post-translational modifications (PTMs) on N-terminal tails of histone proteins (e.g., methylation, acetylation, and phosphorylation). Molecular recognition of modified histones is mediated by ‘reader’ protein subunits. The recruited complexes govern processes such as gene transcription, DNA replication, and chromatin remodeling. Dysregulation of histone modifications and consequent downstream effects have been associated with a variety of disease states, leading to an interest in developing small molecule inhibitors of reader proteins. Herein, we describe a generalized time-resolved fluorescence resonance energy transfer (TR-FRET) assay for a panel of methyl-lysine (Kme) reader proteins. These assays are facile, robust, and reproducible. Importantly, this plug-and-play assay can be used for high-throughput screening (HTS) campaigns, generation of structure-activity relationships (SAR), and evaluation of inhibitor selectivity. Successful demonstration of this assay format for compound screening is highlighted with a pilot screen of a focused compound set with CBX2. This assay platform enables the discovery and characterization of chemical probes that can potently and selectively inhibit Kme reader proteins to ultimately accelerate studies of chromatin reader proteins in normal biology and disease states.

Keywords

TR-FRET; assay development; high-throughput screening; methyl-lysine reader; chromodomain

Introduction

Chromatin structure and dynamics is governed by an interplay of activities including DNA and histone modifications, incorporation of histone variants and nucleosome remodeling, and noncoding RNAs.[1] Regulation of chromatin states through these mechanisms affects

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cellular diversity and plasticity without altering the genetic sequence. Post-translational modifications (PTMs), such as methylation of lysine or arginine residues on the N-terminal tails of histone proteins, are key determinants of chromatin regulation. For example, transcriptionally active gene promoters have enriched levels of histone H3 trimethylation at lysine 4 (H3K4me3), whereas gene promoters that are transcriptionally repressed have enriched levels of histone H3 trimethylation at lysine 9 (H3K9me3) and lysine 27 (H3K27me3).[2] As such, aberrant modification to the PTM landscape has been connected to human diseases and disorders.[1] Because PTMs are inherently reversible, unlike extensive DNA mutations, modulation of chromatin regulation with small molecule inhibitors offers a promising therapeutic strategy for numerous diseases, including cancer.

Recognition of PTMs by proteins with ‘reader’ domains enables further addition or removal of marks *via* recruitment of enzyme complexes that ultimately regulate cellular activities including gene transcription, DNA replication and recombination, and chromatin remodeling.[1] Methyl-lysine (Kme) reader proteins recognize lysine residues that are mono-, di-, or trimethylated at the ε-amino position of the lysine side chain using an aromatic cage binding site, typically formed by two to four aromatic residues (Figure 1A). These aromatic residues engage the modified lysine through cation-π interactions, but hydrophobic interactions and van der Waals contacts also play a role in the energetics of binding the residue.[3] Kme reader proteins achieve selectivity for a specific methylation state or PTM as a result of the size and depth of the pocket as well as the sequence of the adjacent amino acids on the histone tail.

In mammals, the chromodomain is a highly conserved Kme binding motif structurally consisting of a three-stranded anti-parallel β-sheet which folds against a C-terminal α-helix.[4] The human genome encodes 29 chromodomains in total. Eight members belong to the subfamily of chromodomain-containing, chromobox (CBX) proteins which play critical roles in two major transcriptionally repressive complexes: the heterochromatin protein 1 (HP1) proteins consisting of CBX1, −3 and −5, (also known as HP1 $-\beta$, $-\gamma$ and $-\alpha$ respectively) and the Polycomb (Pc) proteins consisting of CBX2, −4, −6, −7, and −8. The HP1 chromodomains are selective for binding to H3K9 methylation whereas mammalian Pc proteins are reported to be more promiscuous *in vitro*, but are mostly associated with H3K27 methylation *in vivo*.[5] Although CBX proteins have been associated with numerous biological functions, deciphering the exact role of each homolog is an ongoing endeavor and requires high affinity and selective inhibitors, also known as chemical probes.[6,7]

Use of small molecule chemical probes is a preferred strategy for investigating and validating the biological functions of specific targets within cells. They are particularly useful for mechanistic studies of biological processes relative to traditional genetic approaches in that they enable modulation of a specific component or activity rather than genetically removing an entire protein from the cellular system. A true chemical probe requires extensive assessment of selectivity, mechanism of action, and cellular activity.[7] Chemical probes can also provide starting points for drug discovery campaigns. For example, efforts on acetyl-lysine reader proteins have led to both high quality chemical probes and molecules suitable for clinical trials.[8] Currently, there are only a limited number

of chemical probes available for Kme reader domains, reinforcing a need for new tool molecules.[3]

A robust, dependable, and reproducible biochemical interaction assay is essential for implementing hit discovery campaigns using high-throughput screening (HTS). Additionally, the ability to accurately characterize inhibitors of a biomolecular interaction is an essential component of developing structure-activity relationships (SAR) to drive hit optimization efforts. Both primary and secondary screening are fundamental requirements for discovering and developing new chemical probes. Criteria considered when choosing a method to employ for these purposes are cost, sensitivity, speed, ease, and reliability.

The AlphaScreen (Amplified Luminescent Proximity Homogeneous Assay; Perkin Elmer) format has been widely used for HTS and characterization of reader protein inhibitors.[9,10] Since AlphaScreen is a bead-based method, it has the particular advantage of detecting weak interactions by virtue of the avidity effect. However, for analysis of competitor compounds, the avidity effect can be a drawback as it potentially can make competitive compounds appear significantly weaker compared to the dissociation constant.[11] Another commonly used method for reader protein assays is fluorescence polarization (FP) using fluorescently labelled histone peptides.[12] One potential issue with the fluorescence polarization assay format is that it often requires high protein concentrations, which results in a higher tight-binding limit for accurately characterizing potent inhibitors. More so, the results of an FP assay using nanomolar concentrations of fluorescently-labeled peptide can be effected considerably by compounds with fluorescent or absorbent properties, leading to fluorescence artifacts.[13] While both of these assay formats have utility for measuring Kme reader protein:peptide interactions and characterizing inhibitors,[6,10,12] we sought a generalizable assay platform that avoids some of the pitfalls of these other techniques.

Here, we report establishment of a homogeneous, non-bead-based approach using TRFRET for Kme reader proteins. This plug-and-play assay platform (Figure 1B) has numerous advantages including solution-based conditions (rather than bead-based), a ratiometric readout, time-resolved fluorescence measurement to reduce compound interference, reduced protein and peptide concentrations (*i.e.* lower tight-binding limit), and the ability to calculate a true K_i under appropriate conditions. This platform utilizes biotinylated tracer ligands and 6X histidine tagged proteins, with europium (Eu)-labeled-streptavidin (donor) and fluorophore-labeled anti-6X-his antibody (acceptor), respectively. The work we describe includes development and utilization of time-resolved fluorescence resonance energy transfer (TR-FRET) assays for chromodomain-containing Kme reader proteins CBX2, −4, −5, −7, −8, CDYL2, and MPP8. In addition, a small, focused screening effort to discover new hit compounds for CBX2 is described. This general assay platform can be easily applied to other members of the reader domain target class toward the discovery of novel ligands.

Materials and Methods

Materials

The LANCE Europium (Eu)-W1024 Streptavidin conjugate and the LANCE *Ultra* U*Light*™-anti-6x-His antibody were obtained from PerkinElmer. Trizma® hydrochloride, Sodium chloride, and Tween 20 were obtained from Sigma-Aldrich. 1,4-Dithio-DL-threitol (DTT) was obtained from Akron Biotech. Assay and dilution plates were obtained from Greiner Bio-One, #784904 and #781280 respectively. The compounds UNC3866 and UNC4195 (Supplemental Figure 1) were synthesized as previously reported.[10] H3K9me3-biotinylated *(ARTKQTARK(Me3)STGGKAPRKQL-K(Biotin)-NH$_2$)* was obtained from the UNC High-Throughput Peptide Synthesis and Array Facility.

Expression and purification of recombinant chromodomain proteins

The chromodomains of CBX2 (residues 9–66 of NP_005180), CBX4 (residues 8–65 of NP_003646), CBX5 (residues 18–75 of NP_036429), CBX8 (residues 8–61 of NP_065700) and MPP8 (residues 55–116 of NP_059990) were expressed with N-terminal His-tags in pET28 expression vectors. The chromodomain of CBX7 (residues 8–62 of NP_783640) and CDYL2 (residues 1–75 of NP_689555) were expressed with C-terminal His-tags in pET30 expression vectors. Proteins were expressed and purified as previously described.[6,10]

General TR-FRET assay conditions

A stock solution of 10X Kme reader buffer (200 mM Tris pH 7.5, 1500 mM NaCl, and 0.5% Tween 20) was prepared, 0.2 μm filtered, stored at room temperature, and was used throughout. Assays were completed using freshly made Kme reader buffer containing 20 mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween 20, and 2 mM dithiothreitol (DTT). White, low-volume, flat-bottom, non-binding, 384-well microplates (Greiner, #784904) were used for assay development and screening with a total assay volume of 10 μL. 384-well, V-bottom polypropylene plates (Greiner, #781280) were used for compound serial dilutions and for transfer of assay mixtures. For compounds with stock solutions in water, serial dilutions were made using Kme reader buffer. For compounds stored in DMSO, serial dilutions were made using DMSO. Following addition of all assay components, plates were sealed with clear covers, gently mixed on a tabletop shaker for 1 minute, centrifuged at 1000 x g for 2 minutes, and allowed to equilibrate in a dark space for one hour before reading. Measurements were taken on an EnVision® 2103 Multilabel Plate Reader (Perkin Elmer) using an excitation filter at 320 nm and emission filters at 615 nm and 665 nm. 615 nm and 650 nm emission signals were measured simultaneously using a dual mirror at D400/D630. TR-FRET output signal was expressed as emission ratios of acceptor/donor (665/615 nm) counts. Percent inhibition was calculated on a scale of 0% (i.e., activity with DMSO vehicle only) to 100% (100 μM UNC3866) using full column controls on each plate. The interquartile mean of control wells was used to calculate Z' values. For dose-response curves, data was fit with a four-parameter nonlinear regression analysis using GraphPad Prism 7.0 or ScreenAble software to obtain IC_{50} values.

Two-dimensional (2D) titration of protein and tracer ligand

A 10 point, two-fold serial dilution was prepared separately for both the protein and biotinylated tracer ligand in a 384, deep-well polypropylene plate using Kme reader buffer. Solutions were made to 3X the final concentration and typically started at 750 nM for protein and 90 nM for biotinylated tracer ligands. Upon addition to the plate, the top final concentrations were typically 250 nM and 30 nM respectively. The tenth well of each dilution only contained buffer for background signal information. A 3X solution of the fluorophore conjugates composed of Eu-labeled streptavidin and anti-6X-His U*Light*-labeled antibody was prepared in Kme reader buffer solution and is referred to as the TR-FRET reagent. 3.3 μL of the protein dilution was added to rows in the assay plate followed by addition of 3.3 μL of the peptide dilution to columns across the assay plate. Lastly, 3.3 μL of the TR-FRET reagent was added to all wells.

Z' and *DMSO tolerance experiment*

Full columns of high signal (no inhibitor, DMSO only) and low signal (100% inhibition with competitor compound; 100 μM UNC3866) were produced to calculate the Z' factor.[14] To complete a DMSO tolerance test, the high and low signals were obtained using concentrations of 0, 0.5, 1, 2, and 3% DMSO. A 1 mM solution of UNC3866 was prepared using Kme reader buffer. Using an automated multi-channel pipette, 1 μL of the inhibitor was added to five columns of the plate to be read for the low signal. Likewise, 1 μL of Kme reader buffer was transferred to five columns for the high signal measurement. 10X solutions of DMSO were made using Kme reader buffer to 0, 5, 10, 20, and 30% DMSO. 1 μL of the DMSO solutions were transferred to both high and low signal columns measurement appropriately. A mixture of protein, biotinylated tracer ligand, and the TR-FRET reagents was made into a single tube. The solution was gently mixed and then aliquoted appropriately to a new column in a deep-well plate. Using an automated multichannel pipette, 8 μL of the mixture was added to the assay plate.

TR-FRET competition assay in dose response format

A 16 point, three-fold serial dilution of compound (UNC3866 or UNC4219) was prepared using Kme reader buffer in a 384, deep-well polypropylene plate. Using an automated multichannel pipette, 3.3 μL of the compound dilution was added to three columns of the assay plate. A mixture of protein, biotinylated tracer ligand, and the TR-FRET reagents was made into a single tube. The solution was gently mixed and then aliquoted to a new column in the deep-well plate. Using an automated multi-channel pipette, 6.7 μL of mixture was transferred from the deep-well plate to the assay plate.

Compound screening using the optimized TR-FRET assay and assay ready plates

For testing compounds in higher throughput, 384-well assay ready plates were prepared in standard plate format: columns 1 and 2 were used for low signal controls (100% inhibition with competitor compound), columns 23 and 24 were used for high signal controls (DMSO only), and columns 3–22 were used for 25 μM single-dose test compounds. Two compound sets maintained in the UNC Center for Integrative Chemical Biology and Drug Discovery (CICBDD) were used: EpiG (960 compounds) and EpiDiamond (64 compounds) where the

compounds generally contain methyl-lysine mimetic features and known epigenetic-related chemical probes. First, controls were added to a mother plate where columns 1 and 2 were filled with 10 mM stock of UNC3866 in DMSO and columns 23 and 24 were filled with DMSO. Test compounds were dispensed across the mother plate at 100X (10 mM) concentration in columns 3–22 using a TECAN Freedom EVO liquid handling work station. Using a TTP Labtech Mosquito® HTS liquid handling instrument, assay ready plates were stamped by stamping 100 nL of control compound into columns 1 and 2, 25 nL of compounds from the mother plate into columns 3–22, and 25 nL of DMSO into columns 23 and 24. Protein, biotinylated tracer ligand, and the TR-FRET reagents were added together and gently mixed by pipetting and rocking. 10 μL was then added to each well of an assay ready plate using a Multidrop Combi (ThermoFisher). Percent inhibition was calculated on a scale of 0% (i.e., activity with DMSO vehicle only) to 100% (100 μM UNC3866) from the full column controls on each plate.

Dose response follow-up for hit validation

To test hit compounds selected for follow-up in dose-response curves, assay ready plates were formatted and prepared similar to the single-dose test plates. Columns 3–12 and 13–22 were used for 10-point serial dilutions of test compounds. For mother plate preparations, test compounds were serially diluted in DMSO 3X across the plate at 100X concentration using a TECAN Freedom EVO liquid handling work station. The top concentration was 10 mM. Using a TTP Labtech Mosquito® HTS liquid handling instrument, assay ready plates were stamped with 100 nL of compound solutions from the mother plate. Protein, biotinylated tracer ligand, and the TR-FRET reagents were added together and then mixed gently by pipetting and rocking. 10 μL was added to each well of an assay ready plate using a Multidrop Combi (ThermoFisher).

Results and Discussion

For target class drug discovery, such as the Kme reader family, development of a screening panel is essential for discovering and evaluating new potent and selective inhibitors and driving SAR studies.[15] TR-FRET is well established as a screening platform for HTS, hit validation, and SAR. The principle of TR-FRET is based on non-radiative transfer of energy between a donor and acceptor fluorophore that results when the fluorophores are within close proximity (~100 Å) of each other. To establish a general TR-FRET method for Kme reader proteins, we employed biotinylated tracer ligands and 6X histidine tagged proteins, labeled with Eu-streptavidin (donor) and fluorophore-conjugated anti-6X-his antibody (acceptor), respectively (Figure 1B). The major advantage of using TR-FRET over other fluorescence methods is the time-resolved detection. Taking a measurement of the emission during the specified delayed time window results in a reduction of signal interference caused by background fluorescence from the plate, buffers, and compounds which occur at the low-nanosecond timescale. This can be particularly advantageous when screening libraries containing auto-fluorescent compounds that may cause interference. The ratiometric calculation of acceptor emission to donor emission also reduces inter-well variations of signal.[13]

To determine the conditions for an optimal TR-FRET signal, titrations of both protein and tracer ligand were carried out in a 10 × 10 (2D-titration) format (Figure 2, Supplemental Figure 2). We typically aimed for a TR-FRET ratio ≥ 3-fold above the background. To develop assays capable of producing SAR for potent compounds (i.e. an assay that has a low tight-binding limit), particular attention was paid to protein/tracer combinations that used the lowest reader protein concentration (Table 1). The TR-FRET assays that we developed for the chromodomains CBX2, −4, −7, −8, and CDYL2 use UNC4195, the biotinylated analog of UNC3866, as the tracer ligand (Supplemental Figure 1). The higher affinity of UNC4195 for the chromodomains versus a biotinylated H3K9me3 or H3K27me3 peptide enables a reduction in the amount of protein and peptide necessary for the assay. A biotinylated analog of the endogenous histone peptide, H3K9me3, was used as the tracer ligand for CBX5 and MPP8, since it binds these two proteins more potently than UNC4195.[10,16] Here, we show that most optimized TR-FRET assays use protein concentrations below 20 nM. (Table 1). The CBX4 and CBX7 assays use lower concentrations of protein and tracer ligand, compared to other proteins tested, to achieve a significant signal because UNC4195 has the highest affinity for these proteins. MPP8 also required less protein to achieve the desired signal because it binds the tracer peptide, H3K9me3, with sub-micromolar affinity.[17] The low affinity of CBX5 for H3K9me3 ($K_d \approx 30$ μM) explains the need for slightly more protein to achieve the desired signal window compared to other readers.[16] To ensure that our assay conditions reach equilibrium, measurements were taken as a function of time and we observed the signal was stabilized after one hour. Therefore, final fluorescence readings were taken following an incubation time between one and two hours.

All of the assays we developed performed well, with Z' values ≥ 0.9, at 1% DMSO (Table 1). We next completed a DMSO tolerance test to determine the maximum solvent limit.[14] With increasing amounts of DMSO, the maximum TR-FRET signals decreased slightly; however, the Z' values remained ≥ 0.9 with up to 3% DMSO (Supplemental Table 1, Supplemental Figure 3). Therefore, DMSO effect on assay performance is practically negligible. For further work using assay ready plates, an appropriate range of final compound concentrations can be obtained while achieving ≤ 1% DMSO (*v/v*) using automation.

To validate these chromodomain TR-FRET binding assays for HTS and demonstrate their utility in finding competitive inhibitors, we performed a series of competition experiments. Assay validation using competitive displacement of the tracer ligand can be completed using the same biotinylated peptide (with addition of excess free biotin after coupling biotinylated tracer ligand to Eu-labeled streptavidin), a non-biotinylated analog of the tracer ligand, or a known inhibitor. For this work, we used UNC3866 as the competitive inhibitor for these TR-FRET reader assays (Figure 2, Supplemental Figure 4), as we have previously determined the binding affinity of UNC3866 for each of these chromodomains by an orthogonal assay.[10] UNC4219, the negative control analog of UNC3866, was simultaneously tested to demonstrate selective displacement of the biotinylated tracer by UNC3866.

An additional advantage of this homogeneous TR-FRET assay is a low tight-binding limit achieved by using low (nanomolar) concentrations of protein. This is in contrast to most fluorescence polarization assays for reader proteins where micromolar protein

concentrations are typically required to achieve appropriate signal with histone peptides as tracer ligands.[6,12] Furthermore, when an assay is developed such that the reagents used are well below the K_d of the ligand, the IC_{50} value can be directly compared to the dissociation constant K_d or K_i. The relationship of IC_{50} to K_i is described by a modified Cheng-Prusoff equation where a protein:ligand interaction is reversible, has 1:1 stoichiometry, and the concentration of protein is significantly lower than ligand concentration (Equation 1).[18] Importantly, the IC_{50} values obtained with our TR-FRET assays (Table 1) match favorably with K_d values reported previously using isothermal titration calorimetry (ITC).[6,10]

$$K_i = \frac{IC_{50}}{(L/K_d) + 1} \quad \text{Eq. 1}$$

We next completed a focused screening campaign with CBX2 in attempt to find new chemical moieties as starting points for designing small molecule inhibitors. Even though this Kme reader has been demonstrated as a therapeutically-relevant target for diseases such as advanced prostate cancer, there are currently no reported potent and selective small molecule inhibitors for CBX2.[19] Our primary screening campaign utilized the EpiG compound sets (maintained in the UNC CICBDD) which includes one set of 960 compounds that are known to bind chromatin modulators or have been produced for chromatin regulatory protein inhibitor efforts and another set of 64 previously described inhibitors for chromatin regulatory proteins. The initial screen of CBX2 with the EpiG sets was completed by testing compounds at a single-dose of 25 μM. The screen was completed in duplicate with consistency in high and low signals and screening statistics (Z' > 0.9) between all plates (Figure 3A, Supplemental Figure 5). The screen of 1024 compounds yielded five compounds with > 50% inhibition and the correlation between all replicates was adequate (R^2 = 0.8) (Figure 3B–C). Nearly 90% of the compounds were distributed within +/− 15% of the DMSO only control (Figure 3D). Only 14 compounds (1.37%) fall outside the −50% inhibition range and are considered to be artifacts. With the exception of one compound, the europium emission at 615 nm for the hit compounds was relatively unchanged. The europiumquenching compound was not investigated further. Our screen produced the hit UNC3866, a known, previously published peptide mimetic inhibitor of CBX2, as the top hit compound with 100% inhibition at 25 μM.[10] The ability to produce UNC3866 as a hit compound validates this assay platform and screening format. In attempt to confirm the other three putative hit compounds, we tested them in a dose-response format. All three compounds produced a dose-response curve yielding weak (3 – 70 μM) IC_{50} values, however we noted that the Hill slopes were > 2.0. Attempts to validate these hit compounds with isothermal titration calorimetry were not successful, therefore we suspect these hit compounds are nuisance hits or artifacts.[20] In fact one of these compounds is very likely a promiscuous inhibitor since it appears as a hit in numerous assays using various detection technologies (historical data within the UNC CICBDD). Even though the TR-FRET format is resistant to many types of fluorescence artifacts, hit compounds using this platform should always be validated with an orthogonal or biophysical assay.

As mediators of active and repressed genomic states, chromatin modifying complexes are important targets to pursue understanding of basic biological function and to conduct drug discovery efforts in numerous therapeutic areas. Producing chemical probes that are selective within families of reader proteins creates opportunities for advancing our understanding of chromatin regulation and also provides information needed to produce more effective therapeutics targeting complex diseases. These efforts are not possible without robust and reliable *in vitro* assays. The panel of TR-FRET assays reported here will support our efforts to develop novel chemical probes for Kme readers. These assays are appealing because they are suitable for either HTS campaigns or quantitative dose-response analysis. Importantly, for chemical probe progression, these assays enable generation of SAR and evaluation of inhibitor selectivity. It is expected that this plug-and-play platform can also be applied to other families of Kme reader proteins such as those containing Tudor, PHD, and WD-40 domains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Kaelin Amaya, Brittany Castellanos, Meenu Immaneni for contributions in the early stages of this project. We also thank Michael Stashko for reviewing experimental data and providing helpful suggestions. Funding for this work was supported by NIH 5R01 GM100919-06, the UNC Eshelman Institute for Innovation, and the UNC Lineberger Comprehensive Care Center.

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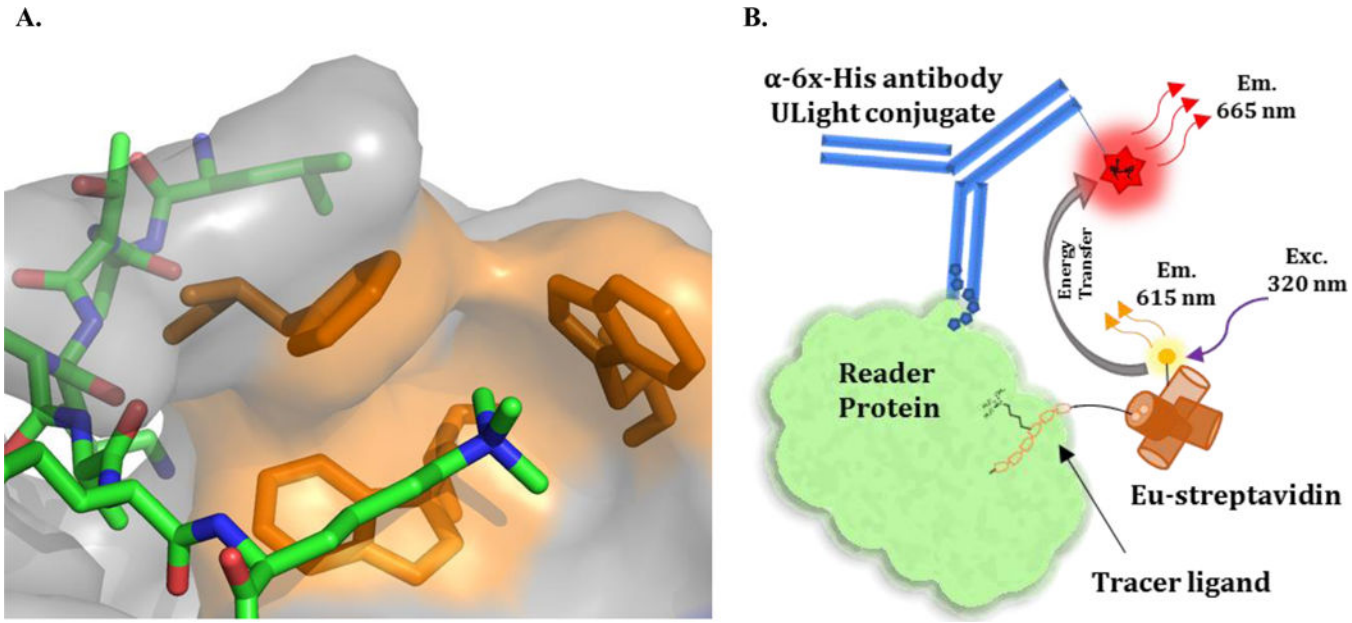

Figure 1.

A) The aromatic cage (orange) of CBX2 surrounding a trimethylated-lysine residue (green). PDB: 3H91 B) Schematic of the Kme TR-FRET assay. The 6X-histidine tagged reader protein and biotinylated tracer ligand are coupled with an anti-6X-histidine antibody and Eu-streptavidin, respectively. Displacement of the tracer ligand with competitor molecule reduces TR-FRET signal.

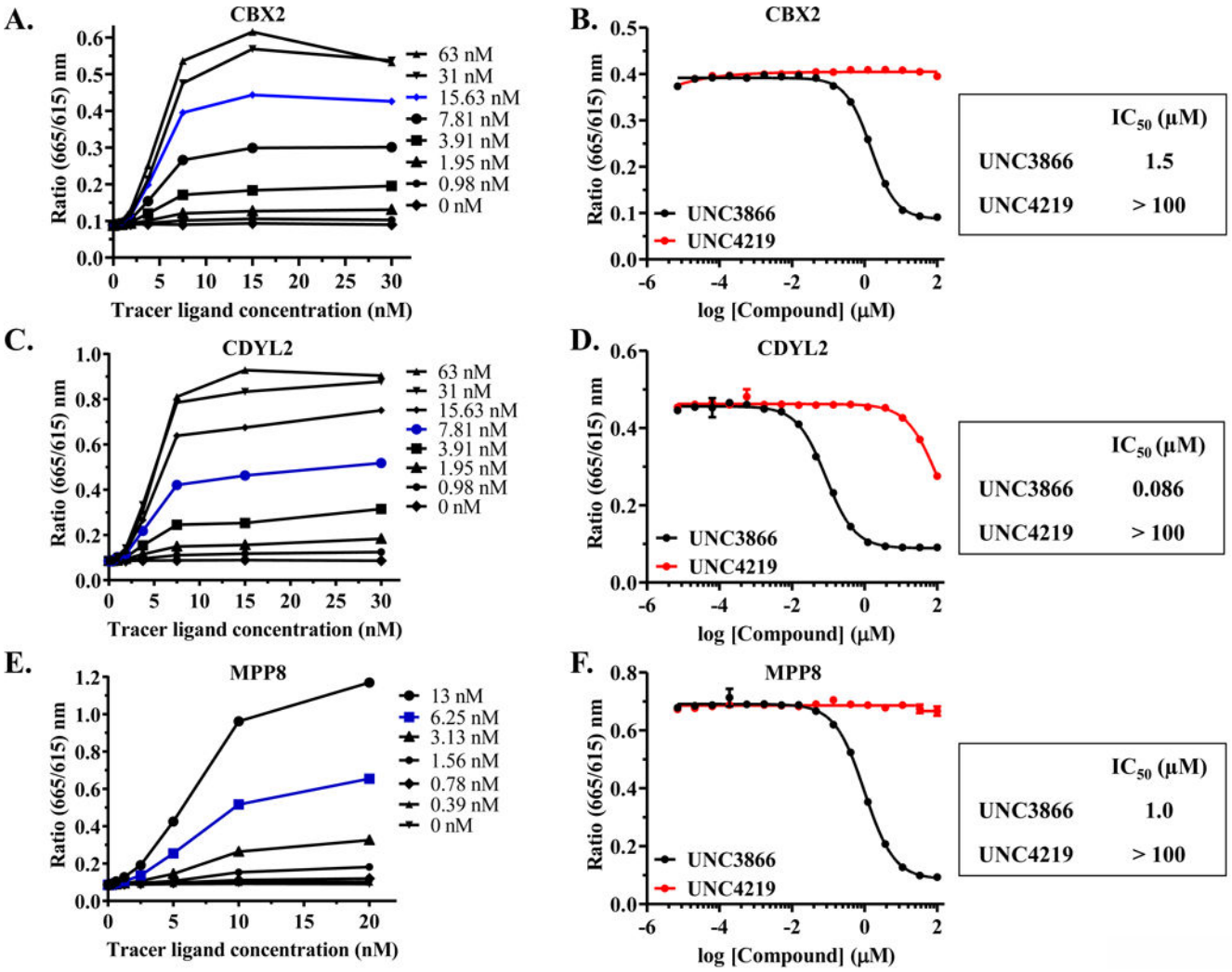

Figure 2.

Examples of 2D titrations for CBX2 (A), CDYL2 (C), and MPP8 (E) with appropriate tracer ligands. The blue line in the 2D titration curve figures is representative of the approximate amount of protein selected to achieve a signal 3-fold greater than the background. Examples of competition experiments using UNC3866 and UNC4219 in dose-response format for CBX2 (B), CDYL2 (D), and MPP8 (F).

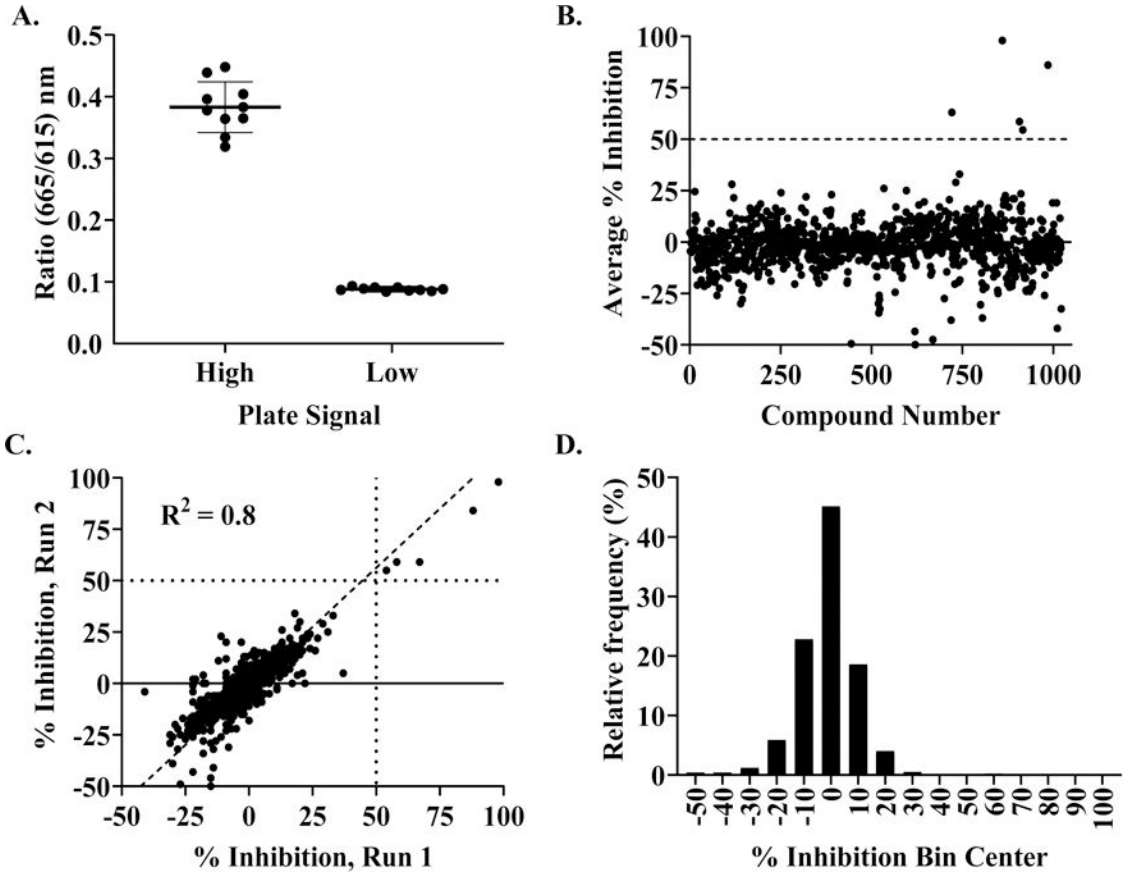

Figure 3.

A) Average high and low signal values for control columns are plotted for each plate in the pilot screen. B) Scatterplot showing the data from the CBX2 pilot screening campaign. Average single-shot inhibition data are shown for compounds from the UNC CICBDD EpiG and EpiDiamond sets that were screened at 25 μM. C) Correlation plot of the compound activities for the duplicate runs. D) Percent distribution related to compound inhibition from the compound sets screened.

Table 1.

Kme reader protein and tracer ligand pairs with finalized concentrations used for TR-FRET assays, the Z' values obtained using those parameters, and the IC_{50} values obtained for UNC3866. UNC3866, at maximum inhibition, was the inhibitor used to obtain the Z' values.

Reader Protein	[Protein] (nM)	Tracer Ligand	[Tracer ligand] (nM)	Z'	IC_{50} (μM)
CBX2	15	UNC4195	30	0.93	1.5
CBX4	5	UNC4195	10	0.94	0.060
CBX5	20	H3K9me3	20	0.97	28
CBX7	1	UNC4195	5	0.95	0.060
CBX8	10	UNC4195	20	0.94	0.56
CDYL2	10	UNC4195	10	0.98	0.085
MPP8	5	H3K9me3	20	0.94	1.0